Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions

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Abstract

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Aim The aim of this study was to evaluate the fate of microorganisms in root canals of teeth with infected pulps and periapical bone lesions with and without the use of calcium hydroxide medication.

Methodology Endodontic samples were cultured and microorganisms were counted and identified in 43 teeth before (sample 1) and after (sample 2) treatment during the first visit and before (sample 3) and after (sample 4) treatment during the second visit. In the first visit teeth were instrumented and half of the teeth were filled with a thick slurry of calcium hydroxide in sterile saline. The other teeth were obturated with gutta-percha and AH-26 sealer. After 4 weeks the teeth with calcium-hydroxide were accessed again and after microbiological sampling they were obturated with gutta-percha and AH-26 sealer.

Results The mean total colony forming unit (CFU) counts of positive samples dropped significantly as a result of canal preparation during the first visit from 1.0×10^6 to 1.8×10^3 (between samples 1 and 2) but increased to 9.3×10^3 in the period between the two visits (sample 2 and 3). There was no difference in mean total CFU counts of positive samples between the end of the first (sample 2) and the end of the second visit (sample 4). The most frequently isolated species were *Prevotella intermedia*, *Capnocytophaga* spp., *Actinomyces odontolyticus*, *Propionibacterium acnes* and *Peptostreptococcus micros*.

Conclusions Although a calcium hydroxide paste was placed in the prepared canals, the number of positive canals had increased in the period between visits. However, the number of microorganisms had only increased to 0.93% of the original number of CFU (sample 1). It is concluded that a calcium hydroxide and sterile saline slurry limits but does not totally prevent regrowth of endodontic bacteria.

Keywords: calcium hydroxide, endodontology, microbiology, root canal.

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Introduction

Bacteria in the root canal system initiate and maintain periapical inflammatory lesions (Kakehashi *et al.* 1965). The bacterial microflora in root canal infections of untreated teeth is dominated by anaerobic bacteria, and several different species are commonly found (Bergenholtz 1974, Byström & Sundqvist 1981, Sundqvist *et al.* 1989, Brauner & Conrads 1995, Le Goff *et al.* 1997). Root canal treatment aims to eliminate bacteria from the infected root canal and prevent reinfection. Cleaning, shaping and irrigating the canal greatly reduces the number of bacteria (Byström & Sundqvist 1981).

However, it has been shown that it is impossible to obtain complete disinfection in all cases, even after thorough cleaning, shaping and irrigation with disinfectants or antiseptics (Byström & Sundqvist 1981, Byström *et al.* 1985, Ørstavik *et al.* 1991). Therefore, concern exists as to the fate and subsequent activity of the remaining microorganisms in the canal. It has been shown that, if the canal is not filled or dressed with a disinfectant

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between two visits, they may multiply rapidly within days, to near the original numbers (Byström & Sundqvist 1981).

It is generally believed that the number of remaining bacteria can be controlled by enclosing an interappointment dressing such as calcium hydroxide within the prepared canal (Byström *et al.* 1985, Chong & Pitt Ford 1992). Some authors therefore consider a two-visit root canal treatment with an interappointment disinfectant dressing mandatory in infected cases (Tronstad 1991). Another approach has been to entomb the remaining microorganisms, depriving them of nutrition and leaving no space to multiply, by the direct and complete filling of the prepared and disinfected canal space in one visit (Soltanoff 1978, Oliet 1983).

Residual bacteria in the apical part of the root canal have been held responsible for failures, even when no bacteria could be detected after the use of an interappointment dressing prior to filling (Sjögren *et al.* 1990, Nair *et al.* 1990). In the present study the number and identities of bacteria in root canals during root canal treatment and the influence of calcium hydroxide on regrowth of bacteria in the period between the first and second visits was investigated.

Materials and methods

Patient selection

Forty-three systemically healthy patients, referred to the Endodontic Clinic of the Academic Centre for Dentistry in Amsterdam for root canal treatment, were selected according to the following criteria. All selected teeth (15 incisors, six canines, eight single-root canal premolars and 13 single-root canal distal roots of mandibular molars) were asymptomatic, did not respond to sensitivity testing, had not received previous endodontic treatment and showed radiographic evidence of periapical bone loss.

The mean age of the participants was 40 years (range 16–86 years). There were 19 females and 24 males. The teeth were randomly divided into two treatment groups. The size of the periapical lesions was determined from the preoperative radiograph by measuring the largest diameter in millimetres.

Microbiological sampling

After cleaning the tooth with pumice and isolation with rubber dam, the crown and surrounding rubber dam were disinfected with 80% ethanol for 2 min. An access cavity was prepared with sterile high-speed diamond burs under irrigation with sterile saline. Before entering the pulp chamber, the access cavity was disinfected again for 2 min with 80% ethanol. Sterility was checked by sampling with a cotton swab over the cavity surface and streaked on blood agar plates. All subsequent procedures were performed aseptically. The pulp chamber was accessed with burs and rinsed with Reduced Transport Fluid (RTF) (Syed & Loesche 1972), which was aspirated with suction tips. RTF was then introduced in the root canal by a syringe with a 27-gauge needle. Care was taken not to overfill the canal. The canal was enlarged to a number 20 Hedström file to the estimated working length as calculated from the preoperative radiograph. Five sterile paper points were consecutively placed in the canal and left for 10 s (sample 1, s1) and then placed in sterile tubes containing 1 mL RTF and transferred to the laboratory within 15 min for microbiological processing.

Endodontic procedure (Table 1)

The working length (1 mm from the radiographic apex) was checked with a radiograph after inserting a size 15 K-file in the canal to the estimated working length, or shorter if the attached electronic apex locator (Apit, Osada, Japan) indicated that the apical foramen had been reached. After the first microbiologic sample (s1), the canal was enlarged using Flexofiles (Dentsply Maillefer, Ballaigues, Switzerland) with the modified double flare technique (Saunders & Saunders 1992), to a master apical file of at least size 35. Each file was followed by irrigation of the canal with 2 mL sodium hypochlorite (2%) in a syringe with a 27-gauge needle. Concentrations of hypochlorite were measured by the titration technique using 0.1 mol L^{-1} $Na_2S_2O_3$ and soluble starch as indicator (Moorer & Wesselink 1982). After preparation, the canal was irrigated with a rinse of 5 mL sodium hypochlorite (2%). Then, inactivation of the sodium hypochlorite was accomplished with a rinse of 5 mL sterile sodium thiosulphate, before a second microbiological sample

Table 1 Endodontic procedure and time of sampling

	Clinical procedure	Group 1 $n = 21$	Group 2 $n = 21$
First visit	microbiological sample 1 (s1)	yes	yes
$t = 0$ week	preparation	yes	yes
(0 days)	microbiological sample 2 (s2)	yes	yes
	obturation	yes	no
	calcium hydroxide	no	yes
Second visit	microbiological sample 3 (s3)	no	yes
$t = 4$ weeks	preparation check	no	yes
(28 days)	microbiological sample 4 (s4)	no	yes
	obturation	no	yes

(s2) was taken from the root canal in the same manner as the first sample.

After drying the canal with paper points, the teeth in group 1 ($n = 22$) were obturated using the warm lateral compaction technique with gutta-percha and AH-26 sealer (Dentsply, Konstanz, Germany). After the first visit all these teeth were restored.

After drying the canal, the teeth in group 2 ($n = 21$) were dressed with a thick mix of calcium hydroxide (Merck, Darmstadt, Germany) in sterile saline. The calcium hydroxide slurry was plugged in the canal with the blunt end of a sterile paper point. If the canal could not be dried, the tooth was excluded from the study. The access cavities in group 2 were filled with two layers of Cavit (ESPE, Seefeld, Germany) and a glass ionomer restoration (Fuji-II, GC Corporation, Tokyo, Japan). In the mandibular molars, the entrance of the distal canal was isolated with Cavit from the remaining pulp chamber in order to prevent contamination by microorganisms from the mesial canals. A radiograph was taken to ensure proper placement of the calcium hydroxide in the canal.

After 4 weeks the patients in group 2 returned. The canal was aseptically accessed under rubber dam isolation and the calcium hydroxide was removed with RTF and careful filing of the canal with the master apical file. Removal of calcium hydroxide from the canal was checked with an operating microscope at 16× (Zeiss, Oberkochen, Germany). A third bacteriological sample (s3) was taken as described previously. After sampling, the canal was rinsed with 5 mL of sodium hypochlorite (2%) and gently instrumented with the master apical file. After inactivation of the sodium hypochlorite with sodium thiosulphate, a fourth sample (s4) was taken from the root canal. The canal was dried and obturated with gutta-percha and AH-26 sealer using the warm-lateral compaction technique. A final radiograph was taken using the paralleling technique with the aid of a beam guiding device (RINN, Rinn Corporation, Elgin, IL, USA). After obturation of the canal, the tooth was restored.

Microbiological procedures

Tenfold serial dilutions of the samples were prepared and 100 μL of each dilution was inoculated on blood agar plates supplemented with 5% horse blood, 5 mg L^{-1} haemin and 1 mg L^{-1} menadione. Plates were incubated anaerobically (80% N_2, 10% H_2, 10% CO_2) at 37°C for 7 days. After incubation, the total colony forming units (CFU) and the different colony types were counted with the use of a stereomicroscope at 16× magnification (Zeiss, Oberkochen, Germany).

All colony types were streaked to purity and incubated aerobically in air with 5% CO_2 (BBL Gaspak CO_2 systems, Becton Dickinson & Co., Cockeysville, MD, USA) as well as anaerobically to determine strict anaerobic and facultative anaerobic growth. Identification was made on the basis of Gram stain, catalase activity and a commercially available identification kit, ATB rapid ID32A (Biomerieux SA, Lyon, France), for strict anaerobes and ATB rapid ID32Strep for aerobic cocci (Biomerieux SA).

In order to allow slow-growing species to develop, the blood agar plates with the total samples were kept under anaerobic conditions from day 7 to day 14. Newly emerging colonies were also streaked to purity and identified.

Statistics

Statistical comparisons were made between groups 1 and 2 for age distribution and size of the periapical lesion using a *t*-test for independent samples. CFU counts, number of strains, number of anaerobes, number of facultatives, percentage of gram-positive rods and cocci and the percentage of gram-negative rods and cocci between group 1 and 2 at the start of the experiment were compared using the Mann–Whitney test for non-parametric data.

Differences between samples 1–4 were compared using the Kruskal–Wallis test for non-parametric data (CFU counts, percentages of gram-positive and gram-negative rods and cocci) or with the ANOVA-test for parametric variables (number of strains, anaerobic and facultative microorganisms). When significant differences were found in the Kruskal–Wallis test, Mann–Whitney tests were performed to demonstrate where the differences were located. When the ANOVA-test showed differences a Scheffé posthoc test was used for the same purpose.

With a positive or negative bacterial sample 2 a *t*-test or Mann–Whitney test, respectively (*) was performed for differences between patients related to gender, age, microbiological differences in CFU count*, number of species, number of anaerobes, number of facultatives, percentages of cocci*, of rods*, and the clinical parameters tooth type, size of radiolucency, preparation length and master apical file size.

P-values <0.05 were considered statistically significant.

Results

One tooth was excluded because the canal could not be dried at the end of the first visit. The radiographs taken after application of the calcium hydroxide all showed that the dressing was well condensed.

Table 2 Proportions (%) and numbers of bacteria recovered from the root canal of teeth with apical periodontitis at the various sampling points

	First visit (n = 42)			
	s1		s2*	
	Mean (SD)	Median	Mean (SD)	Median
% of Gram-negative cocci	12.51(22.64)	0	15 (24.44)	0
% of Gram-positive cocci	34.28 (35.87)	24	42 (45.03)	25
% of Gram-positive rods	12.28 (20.94)	0	18.80 (30.24)	0.5
% of Gram-negative rods	19.60 (30.93)	3	3.3 (8.03)	0
No. of anaerobic species	3.33 (1.60)	3	1.9 (1.37)	1.5
No. of facultative species	0.93 (1.08)	1	0.9 (0.88)	1
No. of total species	4.58 (1.48)	5	2.8 (1.69)	2
CFU mL^{-1}	1.0×10^6 (4.5×10^6)	7.6×10^4	1.8×10^3 (1.1×10^4)	0
No. of positive samples	42		10 (7 group 1)	
	Second visit (n = 21)			
	s3*		s4*	
	Mean (SD)	Median	Mean (SD)	Median
% of Gram-negative cocci	14.33 (24.36)	0	32 (45.25)	32
% of Gram-positive cocci	17.43 (32.20)	0	2.5 (3.54)	2.5
% of Gram-positive rods	13.19 (30.16)	0	4 (5.66)	4
% of Gram-negative rods	14.05 (30.47)	0	38.5 (44.55)	38.5
No. of anaerobic species	0.95 (1.47)	0	3.5 (2.12)	3.5
No. of facultative species	1 (1.1)	1	0.5 (0.71)	0.5
No. of total species	2.1 (1.87)	2	5 (1.41)	5
CFU mL^{-1}	9.3×10^3 (3.1×10^4)	140	1.4×10^2 (4.6×10^2)	0
No. of positive samples	15		2	

SD, standard deviation.
*calculations are related to positive samples only.
s1, s2, s3, s4, sample 1, 2, 3, 4.

Table 2 shows the distribution of bacterial morphotypes at baseline (s1), at the end of preparation in the first visit (s2) and before (s3) and at the end of preparation in (s4) the second visit. There were no significant differences between group 1 and group 2 at the start of the experiment. The age distribution of patients did not differ between groups ($P > 0.05$). Sterility check samples taken before entering the pulp chamber were all negative. Microorganisms were found in all ($n = 42$) initial samples taken from the root canal at the first visit. The median CFU count of the first samples (s1, $n = 42$) was 76 000 (range $80–3 \times 10^7$). An overview of the differences between s1, s2, s3 and s4 are given in Figs 1–3.

Figure 1 represents the mean number of cultivable bacteria in s1, s2, s3 and s4.

After instrumentation and irrigation, the CFU counts at s2 had dropped significantly ($P < 0.05$) to a median of 0 (range $0–7.8 \times 10^4$). This is a reduction to 0.18% of the baseline mean number of CFU at s1 (100%). Thirty-two specimens (77%) had no cultivable bacteria present in the root canal after initial instrumentation. The positive teeth at s2 had harboured significantly more species (5.5 ± 1.7) at the start of the first visit (s1) than the teeth that were negative (4.3 ± 1.4) at s2 ($P < 0.05$). Of the 10 root canals that harboured bacteria after completion of the first visit, seven belonged to group 1 and three belonged to group 2.

At the start of the second visit (s3), the CFU count of group 2 samples was significantly higher ($P < 0.05$) than the counts at the end of the first visit (s2), indicating regrowth of bacteria despite the presence of the calcium hydroxide dressing and a substantial coronal restoration. At s3, the mean number of CFU was 0.93% of the baseline s1. The median CFU count was 140 (range $0–1.4 \times 10^5$). Six (29%) of the 21 teeth in group 2 showed no growth after dressing with calcium hydroxide. The reinstrumentation and final irrigation at the second visit resulted, again, in a significant drop ($P < 0.05$) in median CFU count from s3 to s4. At s4 the number of CFU represented 0.014% of the baseline CFU (s1). Two teeth still harboured cultivable numbers of microorganisms in the root canal at the end of the second visit (s4). Comparisons of the

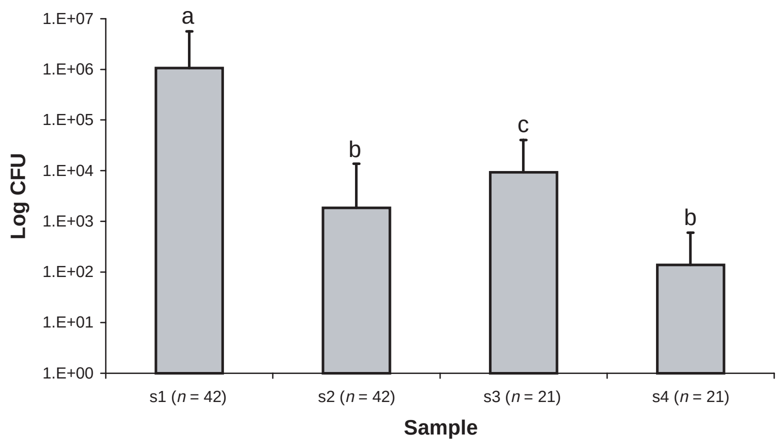

Figure 1 Log CFU per sample. Different letters between samples represent statistically significant differences. Identical letters indicate no statistically significant differences between samples.

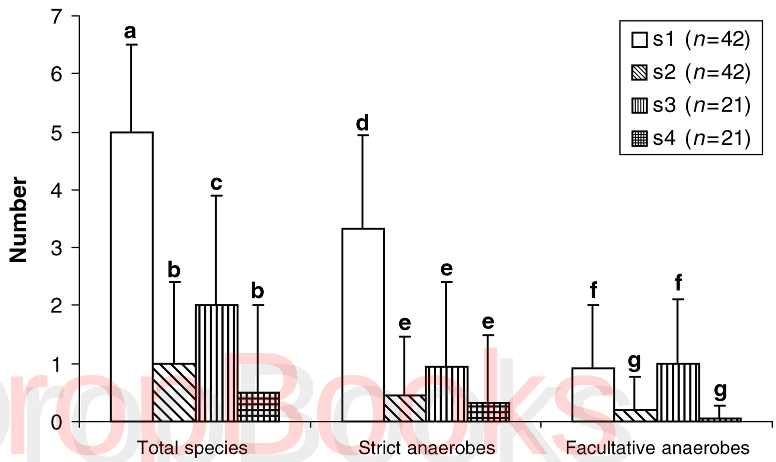

Figure 2 Distribution of strict anaerobes and facultative anaerobes in samples s1, s2, s3 and s4. Different letters between samples (s1, s2, s3, s4) represent statistically significant differences. Identical letters indicate no statistically significant differences between the samples.

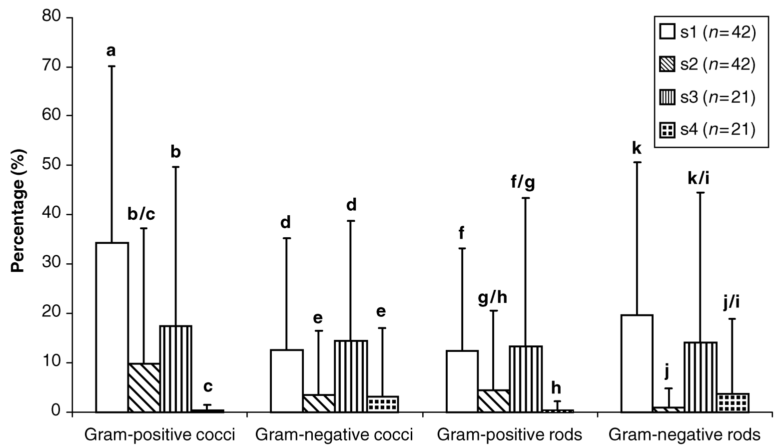

Figure 3 Mean percentage of gram-positive and gram-negative cocci and rods in samples s1, s2, s3 and s4 Different letters between samples (s1, s2, s3, s4) represent statistically significant differences. Identical letters indicate no statistically significant differences between the samples.

median CFU counts between s2 and s4 showed no significant differences ($P > 0.05$) (Fig. 1).

Figure 2 shows the numbers of total species that were strict anaerobic or facultative anaerobic. Figure 3 shows percentages of gram-positive and gram-negative rods and cocci per sample.

The number of CFU ($P < 0.05$), the number of species ($P < 0.05$), the number of anaerobes ($P < 0.05$) and

Table 3 Percentage (no.) of positive root canals harbouring a specific microorganism in samples s1, s2, s3 and s4

	s1 pos $n = 42$	s2 pos $n = 10$	s3 pos $n = 15$	s4 pos $n = 2$
Gram-negative rods	%	%	%	%
Fusobacterium spp.	2(1)			
Fusobacterium nucleatum	12(5)	20(2)	7(1)	
Fusobacterium varium	5(2)			
Fusobacterium necrophorum	2(1)	10(1)		
Fusobacterium necrogenes			13(2)	100(2)
Prevotella oralis	17(7)	30(3)		
Prevotella intermedia	45(19)	50(5)	7(1)	50(1)
Prevotella buccae	10(4)			
Prevotella melaninogenica	5(2)			
Prevotella prevotii	7(3)	10(1)		
Porphyromonas gingivalis	2(1)			
Bacteroides stercoris	2(1)			
Bacteroides thetaiotaomicron	2(1)			
Bacteroides fragilis	2(1)	10(1)		
Bacteroides ureolyticus			7(1)	
Leptotrichia buccalis	2(1)			
Capnocytophaga spp.	17(7)	20(2)	13(2)	50(1)
Gram-positive rods	%	%	%	%
Eubacterium lentum	14(6)	30(3)		
Eubacterium limosum	5(2)	10(1)	7(1)	
Actinomyces spp.	5(2)	10(1)	7(1)	
Actinomyces odontolyticus	29(12)	70(7)	7(1)	50(1)
Actinomyces meyeri	12(5)		7(1)	
Actinomyces viscosis	2(1)			
Bifidobacterium spp.	5(2)		13(2)	
Bifidobacterium adolescentis	2(1)			
Propionibacterium acnes	12(5)	20(2)	20(3)	50(1)
Propionibacterium propionicum	5(2)			
Gram-negative cocci	%	%	%	%
Veillonella spp.	7(3)		13(2)	50(1)
Gram-positive cocci	%	%	%	%
Peptostreptococcus spp.	2(1)			
Peptostreptococcus micros	43(18)	50(5)	13(2)	50(1)
Peptostreptococcus anaerobius	7(3)	10(1)		
Gemella spp.	5(2)			
Gemella morbillorom	7(3)	10(1)	7(1)	
Streptococcus sanguis	2(1)			
Streptococcus oralis			7(1)	
Staphylococcus spp.	5(2)		7(1)	

the percentage of gram-positive cocci ($P < 0.05$) had significantly dropped between s1 and s3. Table 3 shows that the most prevalent bacteria found at the start of treatment (s1) were *P. intermedia* (45%, 19/42 positive samples), *P. micros* (43%, 18/42) and *A. odontolyticus* (29%, 12/42). In the positive s2 samples the same microorganisms were still most prominent, *A. odontolyticus* 70% (7/10), *P. intermedia* 50% (5/10) and *P. micros* 50% (5/10). Although the number of root canals that were positive increased from three to 15 between s2 (group 2) and s3, the number of different species found per positive sample had not increased. At s4 seven different species were isolated from two positive root canals. Four of these species had been present in all four samples, *P. intermedia*, *Capnocytophaga* spp., *A. odontolyticus* and *P. micros*.

Discussion

The presence of bacteria in the root canal system is essential for the development of apical periodontitis and the aim of root canal treatment is their elimination.

The use of Reduced Transport Medium (RTF), the rapid processing of specimens (within 15 min after collection) and the anaerobic techniques used ensured that the microbiological results of the present study were reliable and sensitive. Our results therefore allow comparisons with previous reports where similar microbiological techniques for recovery of microorganisms from the root canal were used.

The present study demonstrated that instrumentation and irrigation with 2% sodium hypochlorite reduced the total number of microorganisms significantly to 0.18% of the original number. Seventy-six per cent (32 out of 42) of the canals had no detectable bacteria after cleaning, disinfecting and shaping in the first visit. Sjögren *et al.* (1991, 1997) found 50–60% negative samples after similar preparation of infected root canals. The difference with our findings could be a result of the different concentration of sodium hypochlorite (0.5% vs. 2%) and different delivery systems used for irrigation. In addition, the difference in sampling techniques and transport media used may have also created differences. The mean numbers of viable bacteria after instrumentation and disinfection (s2) were low in this study, as well as in the studies of Sjögren *et al.* (1991, 1997) ($<10^2$) and similar to the results of Byström & Sundqvist (1981). The median total CFU count of s1 in our study (7.6×10^4) is similar to those of Byström *et al.* (1985) and Sjögren *et al.* (1991), who reported CFU counts of 6.5×10^3 and 9.8×10^4. Byström & Sundqvist (1981) and Ørstavik *et al.* (1991) found CFU counts of 1×10^5–4×10^5 before the start of treatment.

The mean number of species at the start of the first visit was 4.6 (range 2–8). It dropped to a mean of 2.8 (range 2–6) species at the end of the first visit (s2), and did not change after 4 weeks of calcium hydroxide dressing (range 2–6). Indeed, the number of different species per infected root canal was relatively small, and generally ranged between 2 and 8 (Sundqvist 1976, Sundqvist 1992, Brauner & Conrads 1995, Le Goff *et al.* 1997, Sjögren *et al.* 1997, Dahlén & Haapasalo 1998). The percentage of anaerobes (of the total CFU counts) in our study was 97% at the start of the first visit. After 4 weeks of calcium hydroxide dressing the percentage of anaerobes remained at 95%, even though the total number of CFU and the mean number of anaerobic species had dropped significantly. Percentages of 78–93% have been found by others (Bergenholtz 1974, Byström & Sundqvist 1981, Sundqvist *et al.* 1989, Brauner & Conrads 1995, Sjögren *et al.* 1997 and Le Goff *et al.* 1997). Various periods of application of calcium hydroxide dressing have been recommended. Cvek (1973) reported 90% negative cultures after 3 months. Byström *et al.* (1985) reported 100% negative cultures after 4 weeks; Reit & Dahlén (1988) found 74% negative after 2 weeks, Sjögren *et al.* (1991) reported 100% bacteria-free after only 1 week, whereas Ørstavik *et al.* (1991) found 65% of their samples negative after 7 days of calcium hydroxide dressing.

After the application of calcium hydroxide for 4 weeks, we expected the bacterial cell counts (s3) to be similar to or lower than those following preparation of the first visit (s2). However, we found that the median cell count had increased to 140 (range $0–1.2 \times 10^2$), although this represented only 0.18% of the original CFU counts at s1. Ørstavik *et al.* (1991) found that only one of eight positive samples (total of 23 samples) showed higher numbers (5×10^4); from the other seven samples growth was detected but reported as non-quantifiable, meaning very low numbers of bacteria. Although there is some variation, the bacterial cell counts of the 'positive' canals after calcium hydroxide are generally similar in all these studies (most often less than 10^2). One explanation for the differences that do occur could be that remnants of calcium hydroxide are transferred to the microbiological samples, influencing the results. After rinsing with RTF, we consistently visualized remnants of calcium hydroxide in the root canals when checking with the operating microscope (16× using vertical illumination). Before sample s3 was taken, the use of the microscope was essential for complete removal of calcium hydroxide.

Comments have been made previously about the uncertainty of the bacteriological sampling procedure immediately after removal of a calcium hydroxide dressing (Reit & Dahlén 1988). It has been suggested (Reit *et al.* 1999) that microbiological samples should be taken after filling the canal with a sampling fluid (after removal of the calcium hydroxide) for 7 days. However, when the authors applied this procedure culture reversals were seen in both directions. Thus, Reit *et al.* (1999) reported seven canals that turned from a negative to a positive culture after 1 week but also seven canals that changed from a positive culture to a negative culture over the same period. It cannot be ruled out completely that some negative canals in the present study after calcium hydroxide removal (s3), may have become positive if evaluated 1 week later. This means that the results of this study are probably an overestimation of the bactericidal effect of calcium hydroxide. The studies of Reit & Dahlén (1988) and Reit *et al.* (1999) demonstrated the limitations of microbiologic root canal sampling and this should be taken into account when evaluating all root canal procedures and the effectiveness of root canal dressings. Since it was found that removal of calcium hydroxide from the

root canal with the aid of the operating microscope was enhanced and because it has been shown previously that a second culture taken 7 days later did not result in more reliable data (Reit & Dahlén 1988, Reit *et al.* 1999, Molander *et al.* 1990), cultures were taken immediately after removal of the calcium hydroxide. This process was also less demanding for the patients as it reduced the number of appointments.

After irrigation with sodium hypochlorite and gentle filing with the master apical file, s4 showed a median CFU count of 0 ($0–2 \times 10^3$) being significantly lower than s1 but not different from the counts recorded at s2. This indicates that there was no reduction in the number of microorganisms due to the activity of calcium hydroxide. This may be due to the lack of direct contact between the microorganisms and calcium hydroxide in the clinical situation. The pH of calcium hydroxide is approximately 12.5 and this is sufficient to kill rapidly most bacterial root canal species (Byström *et al.* 1985). However, there will always be areas in the canal space and in the root dentine that have low concentrations of hydroxyl ions in solution and where the local pH will be substantially lower (Siqueira & Lopes 1999). Bacteria vary in pH tolerance and most species grow well within a range of pH 6–9 (Padan *et al.* 1981). *Prevotella intermedia*, *Fusobacterium nucleatum* and *Porphyromonas gingivalis* can survive and grow at pH levels between 8.0 and 8.3 (Marsh *et al.* 1993).

Several different species of bacteria are located in the tubules of root dentine (Peters *et al.* 2001). It is difficult to predict the effectiveness of a calcium hydroxide dressing between visits and its ability to destroy the bacterial species located in this area. It is possible that bacteria present in the dentinal tubules escape the direct action of calcium hydroxide (Ørstavik & Haapasalo 1990, Siqueira & Lopes 1999) and are able to (re)infect the canal space. This may explain why root canals at the beginning of the second visit harboured more cultivable bacteria than at the end of the first visit. Another explanation could be that the effect of calcium hydroxide is reduced by dentine (Haapasalo *et al.* 2000).

The most dominant species in this study were *P. intermedia*, *P. micros* and *A. odontolyticus*. Bergenholtz (1974) commonly found species belonging to *Bacteroides*, *Corynebacterium*, *Peptostreptococcus* and *Fusobacterium*. Sundqvist (1992) frequently found *F. nucleatum*, *P. intermedia*, *P. micros* and *P. anaerobius.* Byström & Sundqvist (1981) found the same species to be dominant, whilst Brauner & Conrads (1995) found a high proportion of *P. intermedia*. Positive correlations between *P. intermedia* and *P. micros* and *Eubacterium* were previously reported by Lewis *et al.* (1988), Socransky *et al.* (1988) and Sundqvist (1992). These combinations may be of importance during root canal treatment, since their interrelationship possibly hinders total elimination of microorganisms or provides an environment in which bacteria multiply more rapidly. Of the seven species present at s4 four are frequently found in combination with each other (*P. intermedia*, *Capnocytophaga* spp., *A. odontolyticus* and *P. micros*).

Conclusion

The results of the present study indicate a large reduction of bacteria after instrumentation and irrigation with sodium hypochlorite. A further reduction in the number of microbes was not accomplished by inclusion of calcium hydroxide in sterile saline in the root canal for 4 weeks. However, these conclusions are based on a limited number of teeth.

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